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RNAi-induced off-target effects in *Drosophila melanogaster*

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Supporting Information S1

Scoring scheme to define siRNAs

Description	Score
30%-52% GC Content	1 point
3 or more A/Us at positions 15-19	1 point per A/U
T _m >20 °C	1 point
A at position 19	1 point
A at position 3	1 point
U at position 10	1 point
G/C at position 19	-1 point
G at position 13	-1 point
>4 sequential nucleotide repeat	-9 points
> 4 diplet repeat	-9 points

Scoring scheme used to define most potent siRNAs, based on a summary from several publications. Sequences scoring at least 6 point were considered.

References:

-<http://www.protocol-online.org/prot/Protocols/Rules-of-siRNA-design-for-RNA-interference--RNAi--3210.html>

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- Reynolds, A., Leake, D., Boese, Q., Scaringe, S., Marshall, W. and Khvorova, A. (2004) Rational siRNA design for RNA interference. Nature biotechnology., 22, 326-330.

- Elbashir, S.M., Martinez, J., Patkaniowska, A., Lendeckel, W. and Tuschl, T. (2001) Functional anatomy of siRNAs for mediating efficient RNAi in Drosophila melanogaster embryo lysate. EMBO J, 20, 6877-6888.

dsRNA sequences

CG1559

Atgagcgtggacacgtacgcgccagctcggcgctctcgttcttgacatggacgacaacgagctgcttcgggagcggatactca
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CG2253

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CG3941

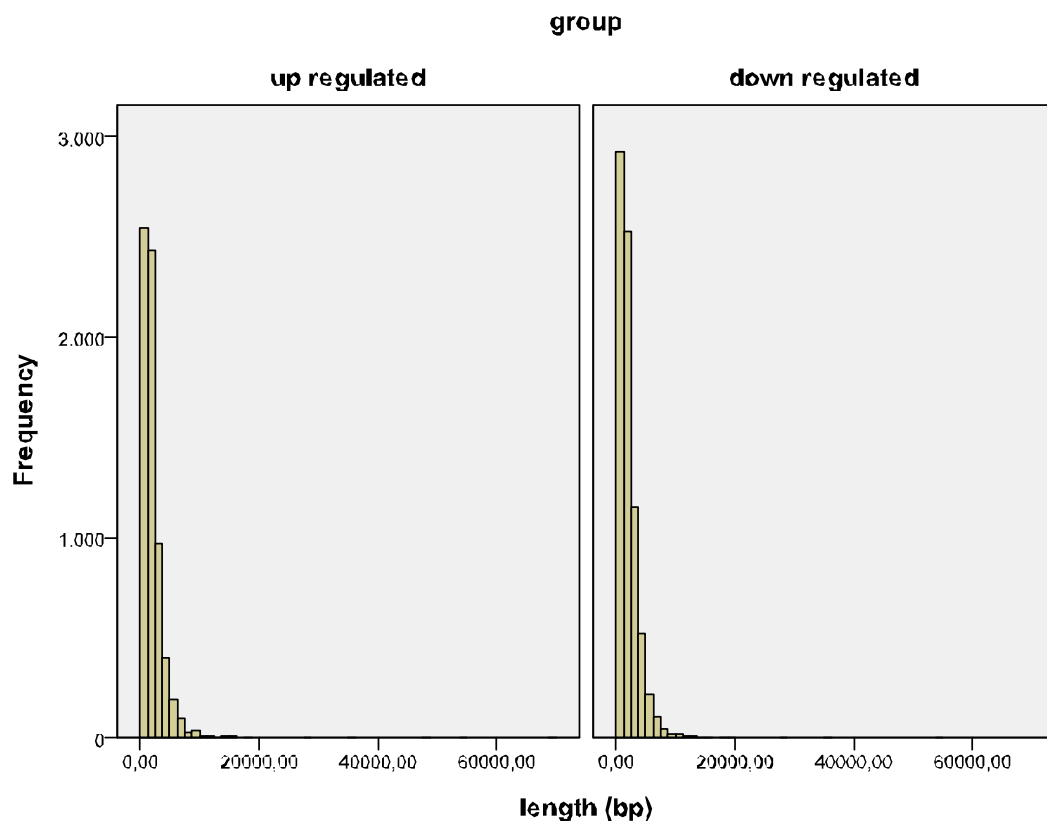
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gcagctccagtgctcctgtgcgaga

The mean length of down-regulated genes is comparable to the length of non-downregulated genes.

A possible difference in length of the genes in the selected off targets genes as compared to the other genes might introduce a bias if there exists a relation between the length of gene and the probability of being down regulated. We analysed this possibility for the array data of CG3941.

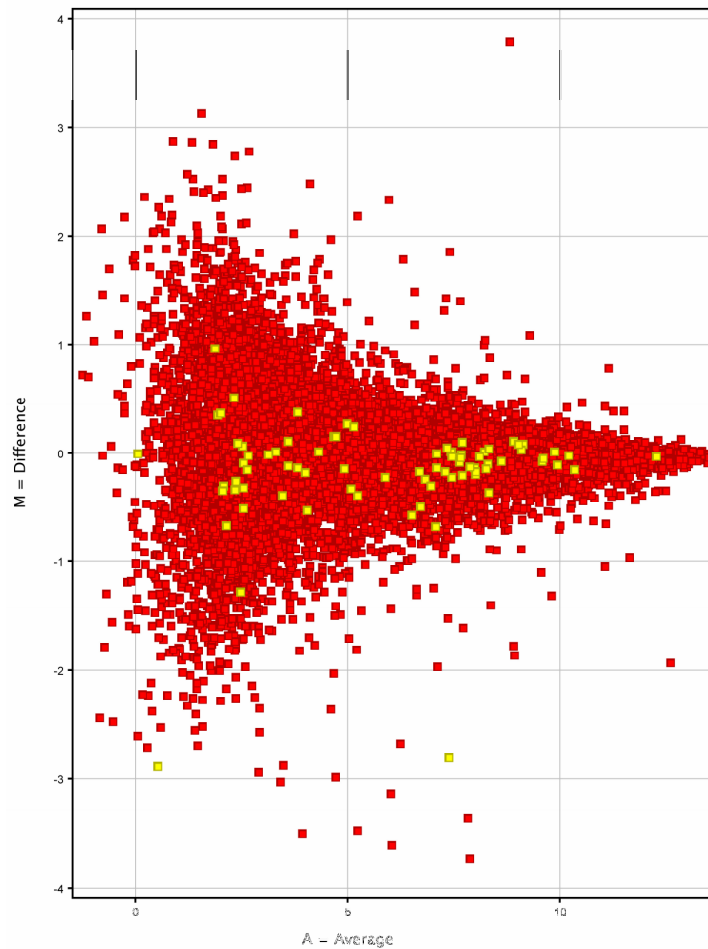
The 7,561 genes which were down-regulated had a mean length of 2078 bp (median was 1588 bp) and the 6,741 genes which were up-regulated had a mean length of 2091 bp (median was 1576 bp).



The distributions of length of the genes for the genes which were up regulated (left side) and the genes which were down regulated (right side).

The Mann Whitney test showed no difference in length of genes between the groups ($P = 0.9$). We therefore conclude that an increase in the proportion of down-regulated genes within the selected group off-target genes is not due to a possible difference in length of the genes.

MA plot of array analysis from CG3941 dsRNA treated cells



MA plot of array analysis from CG3941 dsRNA treated cells (group 1) versus GFP dsRNA treated cells (group 2). The Y-axis shows the probe (log) intensity ratio between group 1 and 2 (M). The X-axis shows the average intensity for the probes (A). Squares denote the individual probes on the array. The yellow squares denote the probes from the genes that are predicted to be an off-target by RNAiSelect. The majority of the squares concentrate on the 0 M-line, which is the result of PLIER normalization (see methods). Most probes chosen by RNAiSelect are found below the 0 M-line (see methods for statistical relevance).